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A NOVEL SEMI-AUTOMATED DIRECT LYSIS METHOD FOR DNA RECOVERY FROM LIVE AND SPENT 9MM AMMUNITION

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Abstract

Recovery of cellular material and DNA from ammunition is a potentially valuable process capable of providing probative evidence of criminal use of firearms. However, DNA profiling success rates on ammunition are low, and consequently much of the ammunition recovered from crime scenes is never submitted for DNA analysis. There is also a common assumption that DNA from fired ammunition is likely to have deteriorated following discharge. In this study, DNA recovery and subsequent STR profiling was conducted on live and spent 9mm ammunition, handled by known donors prior to loading. Two methods were compared; the commonly-used double swabbing technique and a novel semi-automated direct lysis method using AutoLys tubes (Hamilton). The direct lysis method involves placing 9mm cartridges into an AutoLys tube and submerging the cartridge in lysis buffer prior to purification. Lysate was recovered by centrifugation facilitated by the AutoLys tube design. It was found that the direct lysis method recovered significantly more DNA and yielded correspondingly improved STR profiles than the double swabbing technique. It was also shown that DNA could be recovered and profiled using the direct lysis method on both live and spent 9mm cartridges. These results demonstrate that DNA suitable for STR analysis can be recovered from spent ammunition with only slightly reduced yields compared to live ammunition. In many cases the last handler of the ammunition was a major contributor to the recovered DNA. It was also found that the ammunition subjected to the direct lysis method did not have any effects on the ballistic markings imparted on the cartridge during the firing process. This shows the compatibility of the direct lysis method with other traditional ammunition examinations.

Keywords

Ammunition, DNA profiling, ballistic markings.

Introduction

In England and Wales there were a reported 6375 firearm offences, with handguns accounting for over 40% of these [1]. Cellular material deposited on the spent ammunition recovered from crime scenes can be analysed to identify a possible suspect in a case. Currently, such material is commonly collected using a wet and dry double swabbing technique. The profile success rates for ammunition are low and there is also a common assumption that DNA from fired ammunition is likely to be degraded by the heat generated during discharge. Consequently much of the ammunition recovered from crime scenes is not submitted for DNA analysis. In the United Kingdom, the most common ammunition type recovered from crime scenes is 9mm calibre ammunition.

In this study we compare a novel semi-automated direct lysis method using AutoLys tubes for DNA recovery on live and spent 9mm ammunition to the double swabbing method. We also investigate the

effects, if any, this direct lysis method has on ballistic markings and the effects of delayed recovery of ammunition after firing and environmental conditions on DNA persistence of handled ammunition.

Materials and Methods

Brass 9mm (Browning 17mm length) ammunition was used for all experiments. No firearm or ammunition was pre-cleaned prior to usage in an attempt to reflect real casework scenarios. In all experiments, volunteers were asked to handle 9mm ammunition for 10 seconds over the course of a two day period without any prior hand washing.

Experiment One

Volunteers were asked to load the handled 9mm ammunition into a magazine of a 9mm handgun. Half of these (n=20) were subsequently removed from the magazine and placed into AutoLys tubes for DNA extraction using the direct lysis method. The remaining 20 were also removed and placed in sterile universal tubes; DNA was subsequently recovered using the double swabbing method.

Experiment Two

Donors were asked to handle 9mm ammunition of which half (n=25) were fired in a controlled manner at a firing range and the ejected casings were recovered on a plastic sheeting immediately after firing. The remaining 25 were manually ejected from the firearm onto the plastic sheeting and recovered. All fired and unfired ammunition were placed in AutoLys tubes and DNA was then recovered using the direct lysis method.

Experiment Three

Handled 9mm ammunition was fired and either placed inside or outside (unsheltered) for one day, two days or seven days after firing before being collected. DNA was then recovered using the direct lysis method.

For all samples, DNA was extracted and purified using the Investigator STAR Lyse&Prep Kit (Qiagen), quantified using PowerQuant (Promega) and profiled using ESI17 FAST (Promega).

Results and Discussion

Experiment one

The direct lysis method recovered significantly more DNA than the double swabbing method with median DNA concentrations of 3pg/ μ L and 0.2pg/ μ L respectively ($p < 0.05$, Mann Whitney U test). The direct lysis method also recovered significantly more alleles per profile than the double swabbing method.

Experiment two

It was found that DNA could be recovered from both fired and unfired cartridges with significantly more DNA recovered from unfired cartridges ($p < 0.05$, Mann Whitney U test). However, both conditions recovered similar numbers of alleles per profile.

Experiment three

The cartridges fired and left inside for the three time durations showed no significant difference in the amount of DNA recovered and number of alleles recovered per profile. The cartridges left outside showed a decrease in DNA yield and number of alleles recovered when left for seven days after firing compared to the other two time intervals. Outdoor conditions included rainfall on three of the seven days. This suggests that environmental conditions play a larger role in the persistence of DNA on ammunition than time since firing.

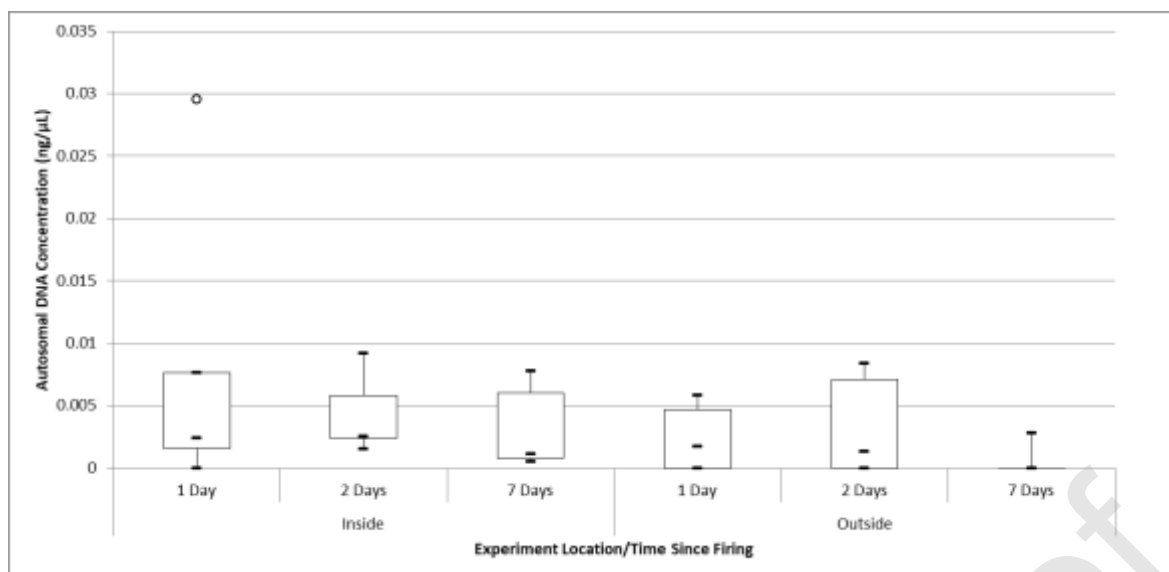


Figure 1. Boxplots comparing DNA concentrations obtained from environmental/time interval experiment using the direct lysis method.

The direct lysis method produced more profiles suitable for loading to the UK National DNA Database (NDNAD) than the swabbing method. Profiles that met the NDNAD load requirements, or which had sufficient alleles designated to be searched without loading were obtained from cartridges left inside and outside, thus improving the evidential value obtained from fired and unfired ammunition.

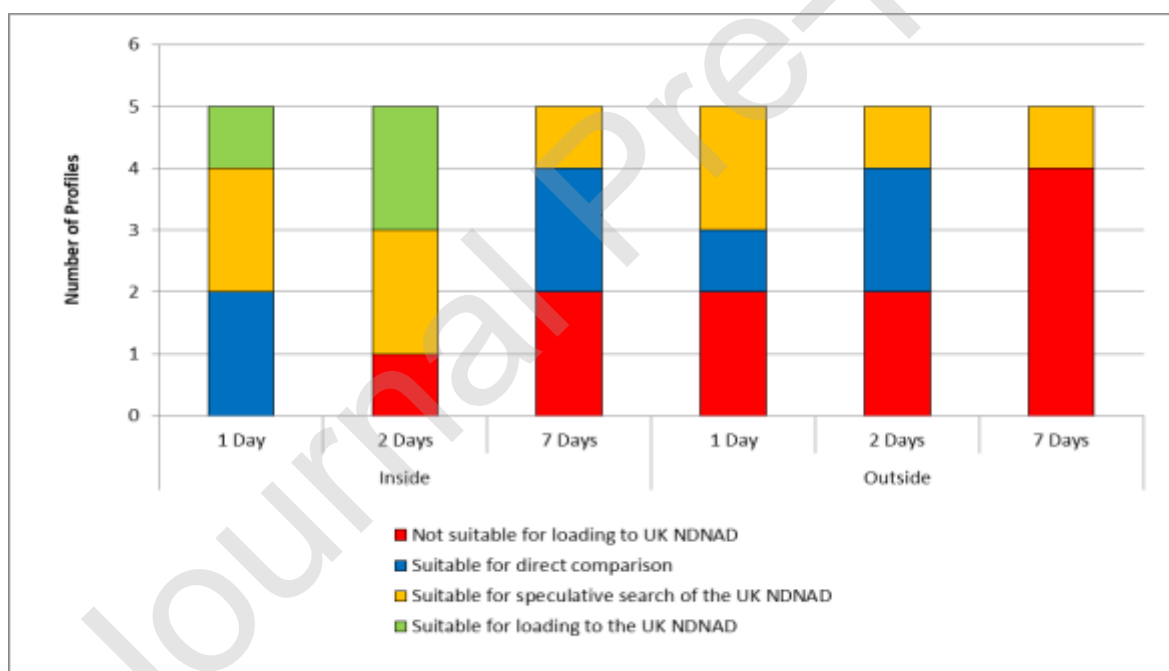


Figure 2. Stacked bar chart showing the number of profiles suitable for direct comparison with a suspect profile; for loading to the UK NDNAD; and for a speculative search on the NDNAD.

It was found that the direct lysis method had no effects on the ballistic markings imparted on the cartridge during the firing process, demonstrating compatibility of the direct lysis method with other traditional ammunition examinations.

Conclusions

1. The direct lysis method is able to recover significantly more DNA from unfired 9mm ammunition than the traditional double swabbing method.
2. Informative DNA profiles were successfully obtained from both fired and unfired 9mm ammunition.
3. DNA profiles could be obtained from fired 9mm ammunition recovered after seven days since firing but may be less informative if subjected to adverse weather conditions e.g. rain.
4. The direct lysis method did not have any effects on the ballistic markings showing the compatibility of this method to traditional ammunition examinations.

References

[1] G. Allen, L. Audickas, Firearm Crime Statistics: England & Wales, (n.d.).
<https://researchbriefings.parliament.uk/ResearchBriefing/Summary/CBP-7654> (accessed February 20, 2019).